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Mini-review

DNA condensation in bacteria: Interplay between macromolecular crowding and nucleoid proteins☆

Renko de Vries*

Laboratory of Physical Chemistry and Colloid Science, Wageningen University, P.O. Box 8038, 6700 EK Wageningen, the Netherlands

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ABSTRACT

The volume of a typical *Eschericia coli* nucleoid is roughly 10^4 times smaller than the volume of a freely coiling linear DNA molecule with the same length as the *E. coli* genome. We review the main forces that have been suggested to contribute to this compaction factor: macromolecular crowding (that "pushes" the DNA together), DNA charge neutralization by various polycationic species (that "glues" the DNA together), and finally, DNA deformations due to DNA supercoiling and nucleoid proteins. The direct contributions of DNA supercoiling and nucleoid proteins to the total compaction factor are probably small. Instead, we argue that the formation of the bacterial nucleoid can be described as a consequence of the influence of macromolecular crowding on thick, supercoiled protein-DNA fibers, that have been partly charge neutralized by small multivalent cations.

1. Introduction

The genomic DNA of bacteria does not spread throughout the whole cell, but instead, is confined into a structure called the nucleoid that occupies only a small fraction of the total cell volume [1]. What are the forces that prevent the DNA from spreading throughout the whole cell? In this contribution, we discuss the relative importance of the various forces that have been suggested to contribute, as well as their interplay, focusing particularly on the interplay between macromolecular crowding and the binding of nucleoid proteins.

At the crudest level, the formation of the bacterial nucleoid can be seen as a (bio)polymer demixing phenomenon [2]. Although living cells are always out-of-equilibrium, the concept of a thermodynamic equilibrium and of a Gibbs energy that is minimal in thermal equilibrium, may still apply in many cases if the cellular system is changing slow enough, as compared to the relaxation time for the equilibrium under consideration. For mixtures of polymers A and B, demixing may be either due to either repulsive or due to attractive interactions between them (as reviewed, for example in [3,4] for food biopolymers. Also see [5]). The case of repulsive A–A, B–B and A–B interactions is usually called "segregative phase separation". In this case, the equilibrium is between two concentrated polymer solutions, one rich in A the other rich in B. For segregative phase separation, the Gibbs energy of the system is reduced by phase separation mainly because since in the demixed state, the polymers have a higher configurational entropy. Practical examples include phase separation in mixtures of globular proteins with flexible, uncharged polysaccharides (for example, see [6]), bundle formation of F-actin, and condensation of DNA induced by flexible polymers ([7–10]).

The case of phase separation in A–B polymer mixtures where the A–A and B–B interactions are repulsive, but the A–B interactions are attractive is usually called "associative phase separation", and the most common example is that of mixtures of oppositely charged polymers. Phase separation now is between a dense complex phase, rich in both A and B, and a supernatant phase, that is dilute in both A and B. For the case of electrostatic complexation the Gibbs energy of the phase separated state is lower, especially because of the increase of the entropy of the counterions that were initially bound to the A and B polymers, but are released upon complexation. Examples of associative polymer phase separation include "complex coacervation" of anionic polysaccharides with basic globular proteins [11], bundle formatin of F-actin filaments induced by polycations [9], DNA condensation induced by polycations [12] etc. Multivalent cations do not really qualify as biopolymers, but also induce condensation and macroscopic phase separation of DNA [13]. The complexation of multivalent cations with DNA can be described as a decrease of the net charge of the DNA. Once the net charge of the DNA falls below some rather low critical value, any small attraction may cause DNA segments to stick

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* Tel.: +31 317 84561; fax: +31 317 48561.
E-mail address: renko.devries@wur.nl.

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together. Exactly what small attraction does the job in which case is a matter of debate: maybe correlation forces [14], bridging attraction (as has been described extensively for longer linear polycations [15]), or even simply van der Waals attraction. The main point is that by taking away most of the net charge of DNA, any small attraction suffices to cause condensation or phase separation.

Besides forces that "push" the DNA into a compact state (segregative phase separation) and forces that "glue" the DNA together (associative phase separation, or DNA condensation by partial charge neutralization), a third main mechanism that contributes to DNA condensation is mechanical stress induced by DNA supercoiling [16], and by the action of DNA binding (nucleoid) proteins that give rise to DNA deformations [17]. In this contribution we make an attempt at a synthesis by considering the interplay between the main forces driving DNA compaction in bacteria: macromolecular crowding, charge neutralization, and mechanical stress. The focus is especially on the interplay between macromolecular crowding and nucleoid proteins.

It is useful to group the molecules inside bacterial cells, with respect to their role in DNA condensation. All of the non DNA-binding macromolecules are the "crowding agents". These are mainly the non-binding globular proteins and RNA. Then there is a group of positively charged "charge neutralization agents" that decrease the effective DNA charge by binding to it. This includes both the small multivalent cations and many (but not all) of the DNA-binding proteins. The DNA binding proteins contribute to condensation via two mechanisms: they may not only decrease the effective DNA charge, but also cause DNA deformation. Finally, for the purpose of this simple classification, DNA supercoiling, which implicitly contributes to condensation by mechanical stress, will be seen as an intrinsic property of the circularly closed bacterial genomic DNA.

First we review the current understanding of some of the individual effects, and then discuss their interplay.

2. Macromolecular crowding

The term "macromolecular crowding" refers to non-specific effects caused by high concentrations of background macromolecules in cells. Various kinds of non-specific effects have been discussed in the literature, especially influences on the rates and equilibrium constant of various kinds of "reactions": binding and unbinding, folding and unfolding [18]. For example, equilibria between different DNA structures are thought to be affected by macromolecular crowding [19]. For DNA condensation, a possibly important indirect effect is that macromolecular crowding may enhance the binding strength of nucleoid proteins [18] and hence influence DNA condensation. In this review we keep these indirect effects in mind, but focus on the direct effect: that of segregative phase separation between crowding agents and the DNA.

2.1. Flexible polymers

The classic model system for DNA condensation induced by macromolecular crowding is the so-called DNA Ψ-condensation of linear DNA discovered by Lerman [7]. This type of DNA condensation is promoted by high concentrations of inert flexible polymers such as poly(ethylene oxide) or Dextran, and high concentrations of (monovalent) salt, whence the name Polymer and Salt Induced DNA condensation. At very low DNA concentrations, the process of macroscopic demixing is very slow, and it is possible to observe individual collapsed coils, for example using fluorescence microscopy [8]. Whereas dilute DNA coils collapse abruptly into very compact globules, the polymer induced condensation of isolated bacterial nucleoids, in which the DNA is supercoiled, is much more gradual [20]. A theoretical analysis of the volumes of the condensed nucleoids as a function concentration of flexible polymers suggests that the isolated nucleoids behave as cross-linked (DNA) polymer gels rather than as free DNA coils.

The origin of the strong salt dependence of DNA Ψ-condensation is the electrostatic repulsion between DNA segments, that opposes condensation, unless screened by the addition of salt. This type of salt-dependence is exactly opposite to that observed for DNA condensation by charge neutralization, where screening by monovalent ions reduces the binding strength of multivalent cations and positively charged DNA binding proteins. DNA Ψ-condensation can be understood in terms of a simple model [10] that we will use in this paper as a framework for discussing the interplay between the different forces driving DNA condensation in bacteria. Part of the simplifications for DNA Ψ-condensation arise because for this case the demixing very often is nearly complete, and it is a fair approximation to neglect the presence of any polymer inside the DNA condensate.[1] This approximation will also be used here.

Suppose we transfer a DNA molecule of contour length L from a dilute solution into a concentrated solution of flexible polymers. The increase in the Gibbs energy associated with the transfer is ΔG_{free}.Very roughly, ΔG_{free} equals the osmotic work needed to push away the flexible polymer segments:

$$\Delta G_{\text{free}} \approx \Pi_{\text{crowd}} V_{\text{excl}} \tag{1}$$

where Π_{crowd} is the osmotic pressure of the crowding agent, in this case the flexible polymer.

The exclusion volume V_{excl} is the volume around the DNA cylinder from which polymers segments are excluded or depleted, as illustrated in Fig. 1. The layer around the DNA cylinder from which the polymer segments are excluded is called a depletion layer in the polymer physics literature. If we introduce an exclusion radius r_{excl} (thickness of the depletion layer), the exclusion volume is

$$V_{\text{excl}} = \pi r_{\text{excl}}^2 L \tag{2}$$

To put this in perspective, a 15 wt% solution of 20 kg/mol polyethylene glycol has an osmotic pressure of about 2.6×10^5 Pa [21]. Assuming an exclusion radius $r_{\text{excl}} \approx 3$ nm, Eq. (1) evaluates to an insertion energy of approximately 7 times the thermal energy $k_B T$, per nm of dsDNA.

In reality, the macroscopic concept of a polymer osmotic pressure does not really apply at lengthscales of the order of r_{excl} (typically a few nm), and this leads to some corrections. Detailed predictions for ΔG_{free} for cylinders immersed in polymer solutions are available from the polymer physics literature [10].

For a mechanical picture of how crowding-induced DNA condensation works, first consider what happens if the depletion layers of neighbouring DNA strands start overlapping, as illustrated in Fig. 2. In this situation, in the region where the depletion layers overlap, there is an unbalanced osmotic pressure: polymers are pushing from the outside, but there are no polymer segments in between the two DNA strands pushing back. Hence the DNA segments will be pushed towards each other. This effective attraction between the DNA segments, caused by the flexible polymers is an example of what is now called a depletion attraction, first proposed by Akasura and Oosawa [22] for particles immersed in

[1] This approximation is not universally valid for DNA condensation by macromolecular crowding: for isolated nucleoids in which the DNA is supercoiled, crowding agent is also present in the condensate, albeit at lower concentrations than outside of the condensate [20].